BIOCATALYTIC OXIDATION OF ACYLGLYCEROL AND METHYL ESTER

GEORGE J. PIAZZA, THOMAS A. FOGLIA, AND ALBERTO NUÑEZ
Eastern Regional Research Center, ARS, USDA,
600 East Mermaid Lane
Wyndmoor, PA 19038, USA

1. Introduction

Soybean lipoxygenase (LOX; linoleate, oxygen oxidoreductase, EC 1.13.11.12) generates fatty acid hydroperoxides from polyunsaturated fatty acids and oxygen. In earlier work the high pH form of soybean LOX was shown to strongly prefer free fatty acid as a substrate, rather than esterified acid (1). However experimental evidence with structural analogues suggests that 15(*S*)-lipoxygenases, such as soybean LOX, recognize and bind their substrates through the methyl end, and therefore modification to the carboxylic end of the substrate should have a minimal impact upon substrate reactivity (2). In support of this contention, recently it was shown that LOX acted upon esterified fatty acids in phosphoglycerides when a bile salt surfactant was present (3-5). Phosphoglyceride oxidation was highly positionally and stereochemically specific and therefore was not merely an enzyme promoted radical-mediated autoxidation (4,5). There is a single report demonstrating the action of LOX upon fatty amide (6).

That phosphoglyceride and fatty amide can be good substrates for LOX raises the question as to whether neutral fatty esters can also be oxidized by LOX. Therefore the susceptibility of acylglycerol and methyl ester toward oxidation by LOX in the presence of the bile salt deoxycholate was measured. These studies are important prerequisites to possible industrial scale processes in which lipoxygenase and other enzymes are used to introduce readily derivatizable oxygen functionality into fats and oils.

2. Results and Discussion

The pH and deoxycholate concentration were varied to determine the optimal oxidation rate of dilinolein (7,8). It was found that the fastest rate of oxidation occurred at pH 8-9 in the presence of 10 mM deoxycholate; critical micelle concentration of deoxycholate: 0.25 - 2.5 mM (9). Having established optimal reaction conditions for dilinolein, other esters of linoleate were tested (Figure 1). Monolinolein was oxidized

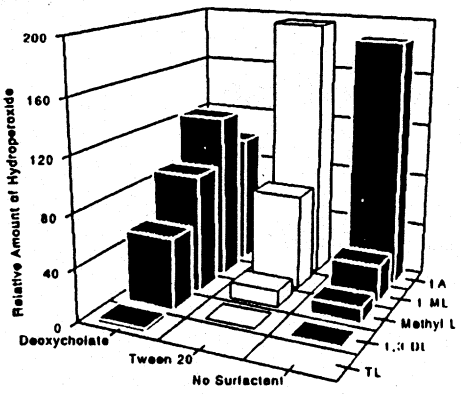

Figure 1. Relative Amount of Oxidized Linoleate Formed by Lipoxygenase in 15 min. LA, linoleic acid; 1-ML, monolinolein; Methyl L, methyl linoleate; 1,3-DL, 1,3-dilinolein; TL, trilinolein.

TABLE 1
Relative Rate of Oxidation of Mixtures of Substrates by LOX in Deoxycholate

Substrate Mixture	Relative Oxidation Rate[a]
1. Linoleic acid	100[b]
1,3-Dilinolein	36 ± 2
2. Linoleic acid	100[b]
Methyl linoleate	39 ± 3
3. Linoleic acid	100[b]
1-Monolinolein	75 ± 2
4. Linoleic acid	100[c]
1,3-Dilinolein	10 ± 2
Methyl Linoleate	18 ± 2
5. Linoleic acid	100[c]
1,3-Dilinolein	36 ± 9
1-Monolinolein	65 ± 4

[a]Relative oxidation rates were calculated by the method of Schellenberger *et al* (10).
[b]Each time point sample contained 75 µg LOX.
[c]Each time point sample contained 113 µg LOX.

more than two times faster than dilinolein and slightly faster than linoleic acid. The methyl ester of linoleic acid was oxidized at approximately the same rate as linoleic acid. In contrast, earlier work that was performed with Tween 20 (polyoxyethylene-sorbitan monolaurate), but without deoxycholate showed that the methyl ester of linoleic acid was a poor substrate for LOX (1). Since the results obtained in deoxycholate were at such variance with these earlier results, assays were repeated in the presence of no added surfactant and in Tween 20. Fig. 1 shows that without surfactant or in the presence of Tween 20 the oxidation of linoleic acid was accelerated, but monolinolein and methyl linoleate were oxidized at rates that were significantly reduced compared to those obtained in deoxycholate. No oxidation of dilinolein could be detected in the presence of Tween 20 or without surfactant.

The data in Table 1 show that when the oxidation of dilinolein, methyl linoleate, and monolinolein was followed in the presence of linoleic acid, their rates of oxidation were reduced relative to that of linoleic acid (Table 1, entries 1-3). However, even in the presence of linoleic acid, monolinolein was still the most rapidly oxidized ester substrate. When 1,3-dilinolein and methyl linoleate were oxidized together in the presence of linoleic acid, their relative rates of oxidation were reduced further, although the amount of LOX was increased proportionately (Table 1, entry 4). The combination of 1,3-dilinolein and monolinolein was also oxidized in the presence of linoleic acid (Table 1, entry 5). Each substrate was oxidized at approximately the same relative rate as before when assayed

separately against linoleic acid.

The data found in Fig. 1 and Table 1 show that the presence of a free carboxyl group on linoleic acid influences the magnitude of its interaction with LOX. In the presence of deoxycholate linoleic acid is oxidized most rapidly in the competition experiments (Table 1), but monolinolein is oxidized most rapidly when the substrates are assayed individually (Fig. 1). If the order of reactivity of the substrates were determined solely by the ability of deoxycholate to solubilize the substrates, then a change in the reaction order would not be expected depending on whether the substrates were assayed separately or together. That linoleic acid is always oxidized most rapidly in the competition experiments must be because of preferential binding of linoleic acid by LOX.

3. Conclusion

In conclusion the results demonstrate that methyl linoleate as well as mono- and diacylglycerol that contain linoleoyl residues are rapidly oxidized by LOX in buffers that contain deoxycholate surfactant. The oxidized products are useful chemical intermediates for further transformation to emulsifiers, coatings, and lubricants with enhanced hydrophilic properties.

4. References

1. Christopher, J., Pistorius, E., and Axelrod, B. (1970) Isolation of an isozyme of soybean lipoxygenase, *Biochim. Biophys. Acta* 198,12-19.
2. Lehmann, W.D. (1994) Regio- and stereochemistry of the dioxygenation reaction catalyzed by (S)-type lipoxygenases or by the cyclooxygenase activity of prostaglandin H synthases, *Free Radical Biol. Med.* 16, 241-253.
3. Eskola, J. and Laakso, S. (1983) bile salt-dependent oxygenation of polyunsaturated phosphatidylcholines by soybean lipoxygenase-1, *Biochim. Biophys. Acta* 751, 305-311.
4. Brash, A.R., Ingram, C.D., and Harris, T.M. (1987) Analysis of a specific oxygenation reaction of soybean lipoxygenase-1 with fatty acids esterified in phospholipids, *Biochemistry* 26, 5465-5471.
5. Therond, P., Couturier, M., Demelier, J.-F., and Lemonnier, F. (1993) Simultaneous determination of the main molecular species of soybean phosphatidylcholine or phosphatidylethanolamine and their corresponding hydroperoxides obtained by lipoxygenase treatment, *Lipids* 28, 245-249.
6. Ueda, N., Yamamoto, K., Yamamoto, S., Tokunaga, T., Shirakawa, E., Shinkai, H., Ogawa, M., Sato, T., Kudo, I., Inoue, K., Takizawa, H., Nagano, T., Hirobe, M., Matsuki, N., and Saito, H. (1995) Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist, *Biochim. Biophys. Acta* 1254, 127-134.
7. Piazza, G. J. and Nuñez, A. (1995) Oxidation of acylglycerols and phosphoglycerides by soybean lipoxygenase, *J. Am. Oil Chem. Soc.* 72, 463-466.
8. Nuñez, A. and Piazza, G.J. (1995) Analysis of lipoxygenase kinetics by high-performance liquid chromatography with a polymer column, *Lipids* 30, 129-133.
9. Murada, Y., Sugihara, G., Nishikido, N., and Tanaka, M. (1982) Study of the micelle formation of sodium deoxycholate, in K.L. Mittal and E.J. Fendler (eds.), *Solution Behavior of Surfactants*, Plenum Press, New York, pp. 611-627.
10. Schellenberger, V., Siegel, R.A., and Rutter, W.J. (1993) Analysis of enzyme specificity by multiple substrate kinetics, *Biochemistry* 32, 4344-4348.